

KERATIN AND RELATED GENES IN THE INVESTIGATION OF THE STRUCTURAL BASIS OF WOOL QUALITY AND ITS IMPROVEMENT

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INTRODUCTION

Man-made fibre structure compared with wool structure

In examining approaches that research can take to improving wool quality it is instructive to consider some crucial features of man-made compared to wool fibres. Man-made textile fibres are composed of polymer molecules (very large and very long) that are uniform in chemical composition. In the synthetic fibre industrial process, the technologist can utilise known chemical and physical properties of the raw material in a *predictive* manner to produce a fibre with a specified diameter, length, strength, elasticity, dyeing properties and so on.

Wool fibres, on the other hand, are not continuous filaments of uniform structure like a man-made fibre. Their polymer is composed of a complex mixture of proteins packaged into cellular compartments that stick tightly together. Since the wool polymer is produced by a biological factory, the follicles, it is influenced profoundly by genetics and environmental factors that interact in the sheep in numerous subtle ways. These biological factors are the origin of wool quality but at the same time their complexity makes the technological job of quality improvement more difficult than with the man-made synthetic product.

Wool quality through selective breeding technology

Modern wool properties are the result of quantitative genetic selection over many decades of natural mutations affecting fibre diameter, cell distribution and wool keratin composition. The researchers and breeders utilise data derived from measurements of wool properties such as variations of diameter and strength in sheep lines. The objective is an improvement in a phenotypic character unaccompanied by a less favorable character for example, achieving lower diameter without decreased fleece weight. In this conventional approach the genes being manipulated are not known nor is it known whether the gene products have their effect in the fibre structure or in the follicle. There will be a practical limit to what improvements can be achieved by breeding strategies, a limit imposed by the genes that are present in the sheep population.

WOOL QUALITY THROUGH TRANSGENIC TECHNOLOGY

Transgenesis can transcend the barrier presented by the limits of the gene pool. In selective breeding for a particular character such as intrinsic fibre strength, the genes involved are unknown and where they act is not known. In contrast, sheep transgenesis begins at the opposite end of the gene-wool properties relationship. Many genes are now known that encode the proteins of the wool polymer and have been characterised as sequences of nucleotides, the building blocks of DNA.

Table I is a summarised list of the many and various proteins identified in wool. In detail the list is more extensive and there are still more proteins and their genes to be identified, however, genes representative of the different families of proteins have been characterised. Where these genes function in the follicle is accurately known from extensive mapping of gene expression in wool follicles using radioactive probes and *in situ* hybridization (Powell et al. 1991, Powell & Rogers, 1994) and electron microscopy, and the information is diagrammatically represented in Fig.1. Thus the IF genes are the first to be expressed and the proteins begin depositing just above the bulb. This is followed by the matrix proteins, the genes of the sulphur-rich group being the last to be expressed. Although these processes begin at different levels in the follicle they eventually overlap to a large extent so that for example, intermediate filament proteins are being laid down at the same time as the matrix proteins.

WOOL KERATIN PROTEINS AND THEIR GENES			
Group	Family		No. of Genes
IF (filaments-cortex)	IF type I	(IF)	4-5
	IF typeII		4-5
IFAP (IF-associated proteins of the matrix)	High-sulphur B2	(HS)	7
	High-sulphur BIIIA		11
	High-sulphur BIIIB		4
	Ultra-high-sulphur/cortex	(UHS)	10
	Ultra-high-sulphur/cuticle		6
	High-glycine/tyrosine type I F	(HGT)	1
	High-glycine/tyrosine type I C2		1
	High-glycine/tyrosine type II		10

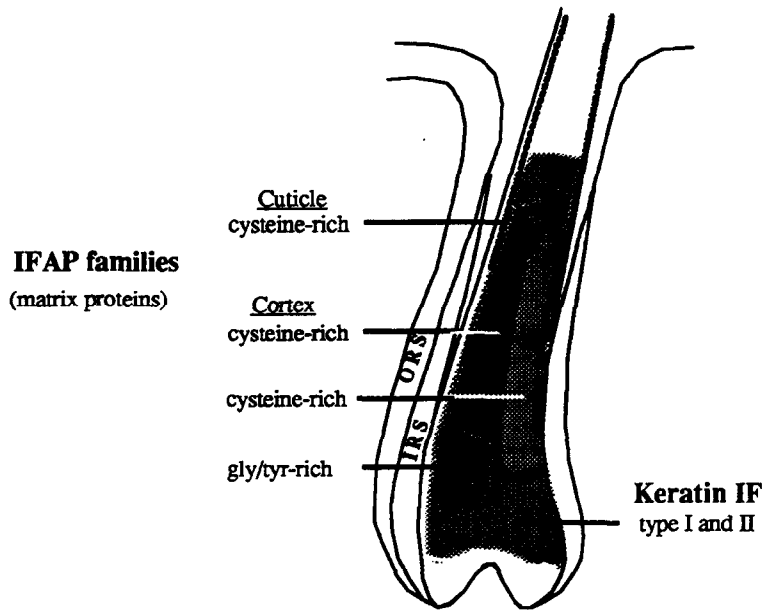


Figure 1. Diagram illustrating the different levels at which the various keratin gene families are expressed in the wool follicle. The abbreviations are as given in Table 1.

Many years of research on the structure of wool fibres, combining electron microscopy with biochemical identification of the proteins, have led to our present knowledge of where the keratin proteins are located in the wool fibre structure. This is shown in the electron micrographs of Figs. 2 and 3. Figure 2 is a high resolution picture within part of a cortical cell showing the intermediate filaments in profile and the location of the matrix surrounding them. Together they constitute the filament-matrix complex. The filaments are the origin of the elastic behaviour of wool fibres but the ultimate strength of the fibre also relates to the molecular connections between the matrix and the IF proteins. Virtually nothing is known about this aspect of the structural basis of wool properties.

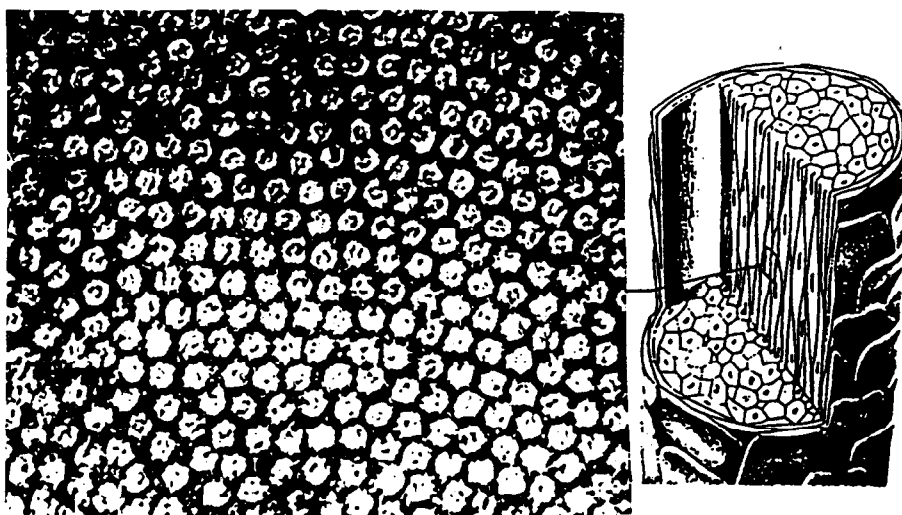


Figure 2. On the left is an electron micrograph of part of a cortical cell in cross-section. The intermediate filaments are about 8nm in diameter and are closely packed surrounded by the densely-stained matrix. X 500,000. On the right is a diagram to illustrate the cortical cells in the fibre that contain the filament-matrix complex.

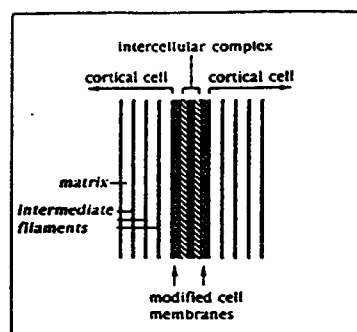


Figure 3. On the left is an electron micrograph of a cross-section of a wool fibre showing portions of several cortical cells and the the cell membrane complex (m) separating them. X 90,000. On the right is a diagram showing in longitudinal array, the major components discussed in the text.

The other component of wool structure, the intercellular membrane complex which also plays a crucial role in holding the wool fibre together is shown in Fig. 3. The strength of a wool fibre must ultimately depend as much on the integrity of the adhesion that resides within this laminated structure as it does on the filament-matrix complex and yet we know so little about its chemical constitution.

Wool structure is understood in broad outline as given above but there is still much to be learned about how the keratin proteins are arranged, how they interact with one another at the molecular level and the relationship between their abundance and distribution to the mechanical properties of wool. A better understanding here is vital for the future manipulation of fibre properties and the transgenic route offers a direct means for investigating these questions systematically. Therefore, if individual genes can be selected, constructed by molecular genetic techniques into a transgene and introduced into sheep, the composition and structure of the wool fibre in principal, should be dramatically altered through over-expression of the gene and changes in properties evaluated. Both the cortex and the cuticle are candidates for manipulation by selected keratin transgenes. The essential feature of a transgene is that it must contain a promoter region that enables the coding part to be targeted to the follicle in the first place and secondly, for the protein product to be finally located in the required location of the fibre. The most useful promoter that has been studied so far is one that controls a filament gene of the cortex. The gene has been called K2.10. The K2.10 promoter has the required specifying properties and several transgenes have been constructed using this promoter and are currently being studied. An outline of the transgene structure is shown in Fig. 4.

THE K2.10 PROMOTER - TRANSGENE

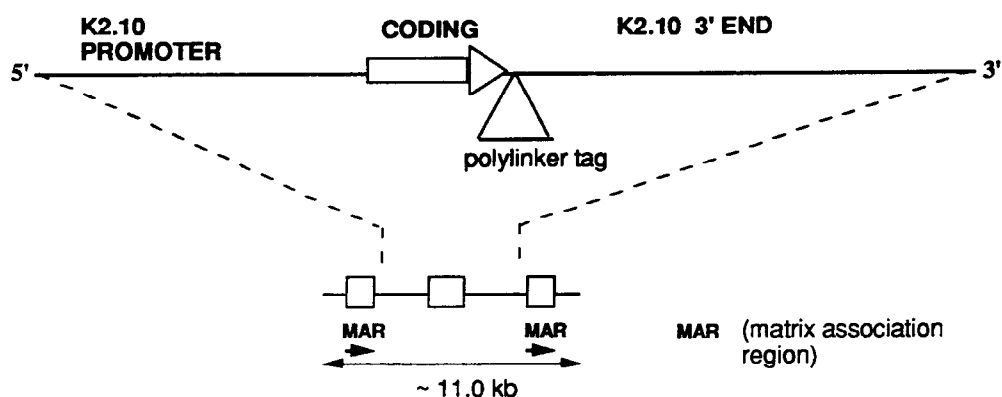


Figure 4. Outline of a transgene construct showing the arrangement of the coding region of a keratin protein behind a promoter which will drive over-expression of the encoded protein specifically directed to the fibre cortex. The MARs refer to "matrix attachment regions" that are special DNA sequences that are being tested for their ability to assist in regulating the level of expression of the encoded protein. In order to save time, transgenic mice are usually used for testing gene constructs prior to application to sheep.

In addition to increasing the abundance of a keratin protein the techniques can be modified so as to *decrease* the abundance of that protein or a promoter from one of the keratin genes can be chosen to express another keratin gene in an abnormal location in the fibre structure. For example, a protein that is normally found in the fibre cuticle has been expressed at a high level in the cortex and it deposits in the

cells as large aggregates of protein like those that form naturally in the cuticle cells (Fig. 5). The effect of this particular result on fibre mechanical properties has not yet been determined. The experimental strategy of accumulating data linking specific proteins or combinations of them to wool structure and mechanical properties should eventually be of predictive value for wool quality.

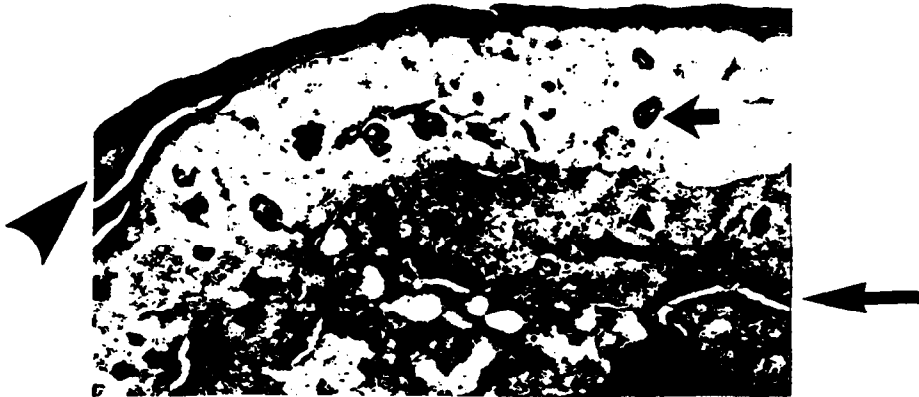


Figure 5. Electron micrograph of several cortical cells of wool in cross-section with deposits of cuticle protein (smaller arrow) produced by driving a gene encoding a cuticle protein with the cortex-specific promoter, K2.10 in a transgenic sheep. Some of the cortical cells appear to be denser than normal (long arrow). The cuticle (arrowhead left) appears normal.

Sheep transgenesis experiments currently in progress and planned for the future are directed to:

- (1) altering the ratio of filaments to matrix
- (2) changing the protein composition of the matrix itself and
- (3) introducing a cross-link other than the disulphide bond between the protein chains. In the first case the elasticity of wool could be expected to be increased if more filaments were present in the cortical cells and fibre strength might also be increased. To achieve this (Fig. 6) the two genes for type I and type II proteins will be driven by the same promoter (K2.10) to optimise the likelihood of their co-expression in equal amounts. The equivalent amounts are necessary because that is the ratio in which they occur in the filaments.

Increasing the proportion of intermediate filaments

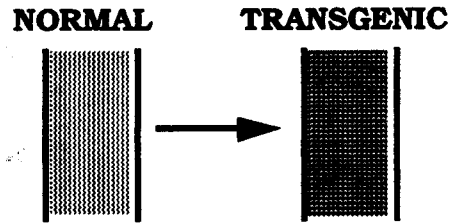


Figure 6. Increasing the concentration of intermediate filaments in the fibre to alter its elastic properties.

The second type of transgenesis experiment is based on the fact that fine wool fibres have a bilateral structure in which the cortex consists of two segments, the orthocortex and the paracortex (Mercer, 1953; Fraser & Rogers, 1955). The cells differ markedly in arrangement of the filament and matrix components. Further, the protein composition of the paracortex matrix has more sulphur-rich proteins than that of the orthocortex (Jones et al. 1993) whereas there is a higher concentration of glycine and tyrosine-rich proteins in the matrix of orthocortical cells (Fratini, et al. 1994; Jones et al. 1993). Genes for proteins of either type could be over-expressed but the first to be tested will be over-expression of a glycine/tyrosine gene in an attempt to convert the paracortex so that the most or all of the fibre cortex becomes ortho-like (Fig. 7). The transgenic wools will be examined for any changes in mechanical properties.

Increasing orthocortical cell characteristics

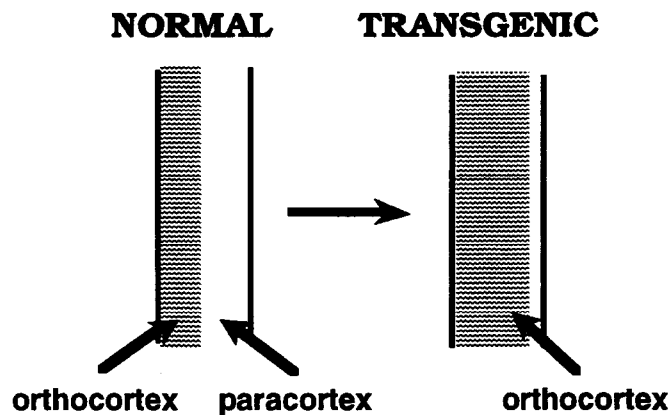


Figure 7. Increasing the proportion of orthocortex-type cortical cells by increasing the expression of a high glycine-tyrosine matrix protein gene in the cortex.

The third type of experiment is to introduce a gene that will give rise to expression of the enzyme transglutaminase in the cortical cells. (Fig 8). The activity of this enzyme in the developing cortex of the growing fibre should produce new crosslinks different from and in addition to the disulphide bond. This isopeptide chemical bond, present in the cortex in only trace amounts, could be expected to form between the protein chains of the filaments and of the matrix and also between filaments and matrix. Expected consequences of this could be an increase in intrinsic fibre strength although the elasticity of the fibre might be decreased.

Isopeptide crosslinks added to the normal content of disulphide bonds

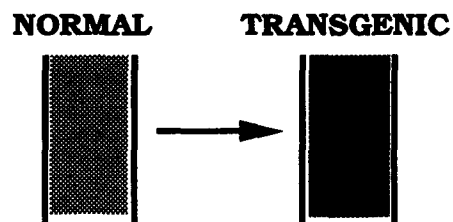


Figure 8. Diagrammatic representation of increasing the crosslinking between keratin protein chains by the introduction of isopeptide links. To achieve this, the enzyme transglutaminase has to be expressed in the cortical cells in an active state to catalyse the chemical reaction of cross-linking.

SUMMARY

The potential of sheep transgenesis for wool quality is to be able to take a particular known gene or set of genes and produce a sheep growing wool with specific and predictable properties. Clearly, these genes must be accurately targeted to express in the wool follicle and not elsewhere in the sheep, a potential disadvantage were it to be so. In Adelaide we have demonstrated that the technique can be made to work correctly in this respect and experiments are now underway to develop its application.

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